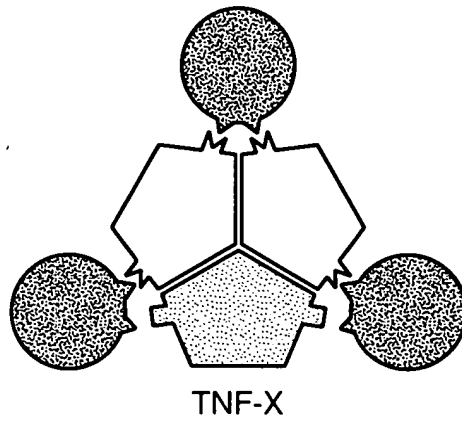


**FIG. 1A**



**FIG. 1B**

2025-06-26 10:00

CU3E1U-032E13600

TNF-TNFR TRIMER COMPLEX

TOP VIEW

SIDE VIEW

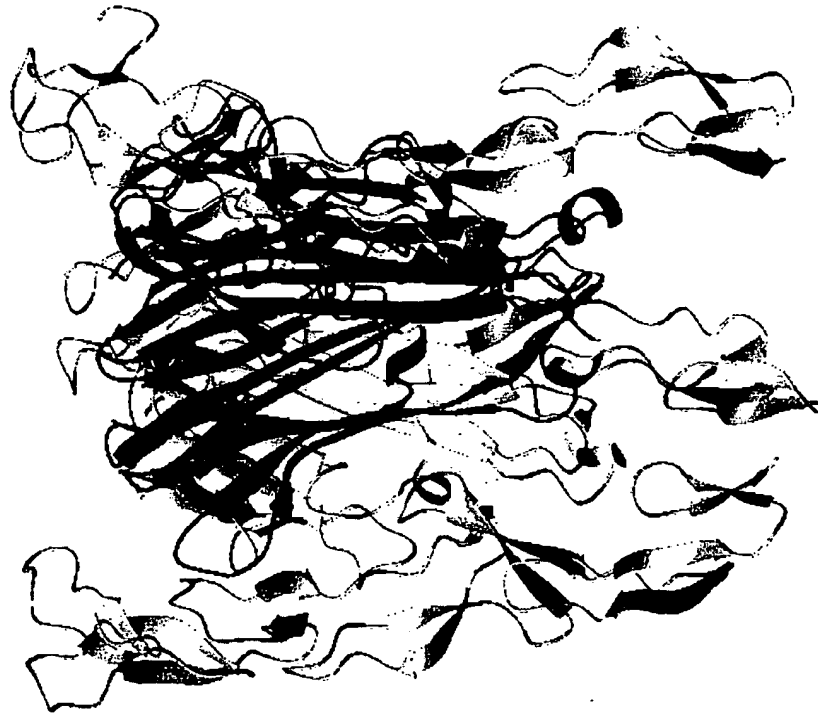
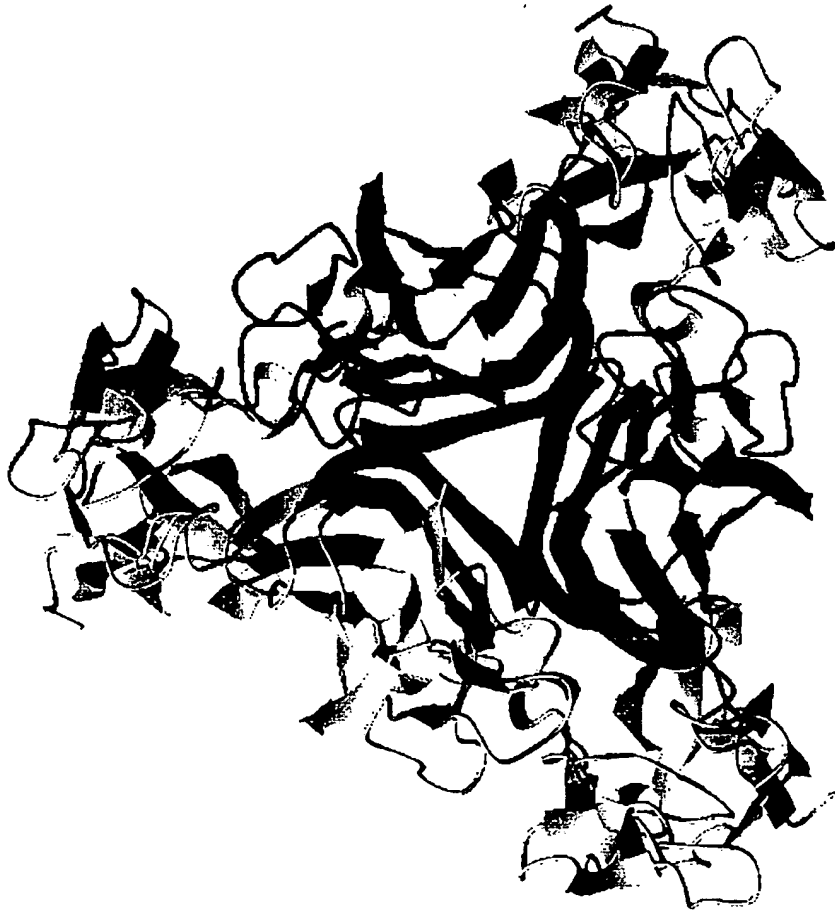
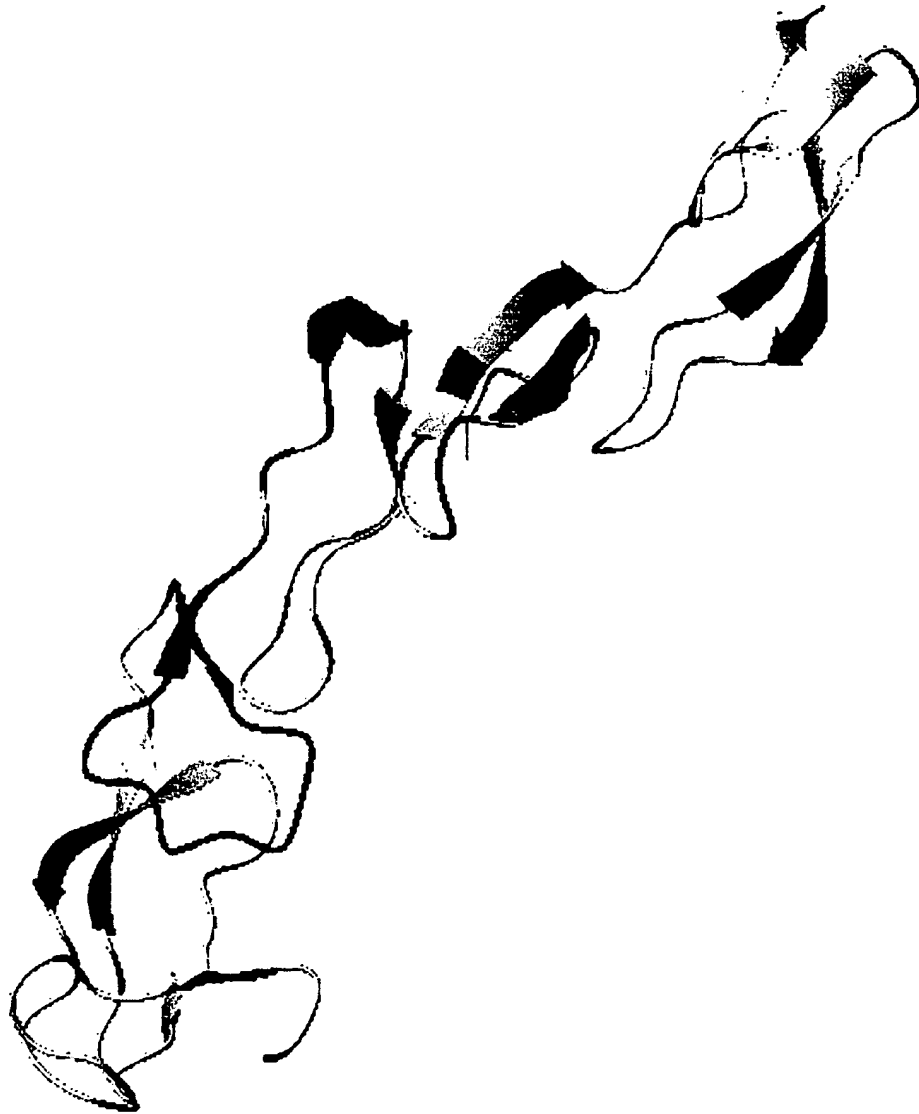
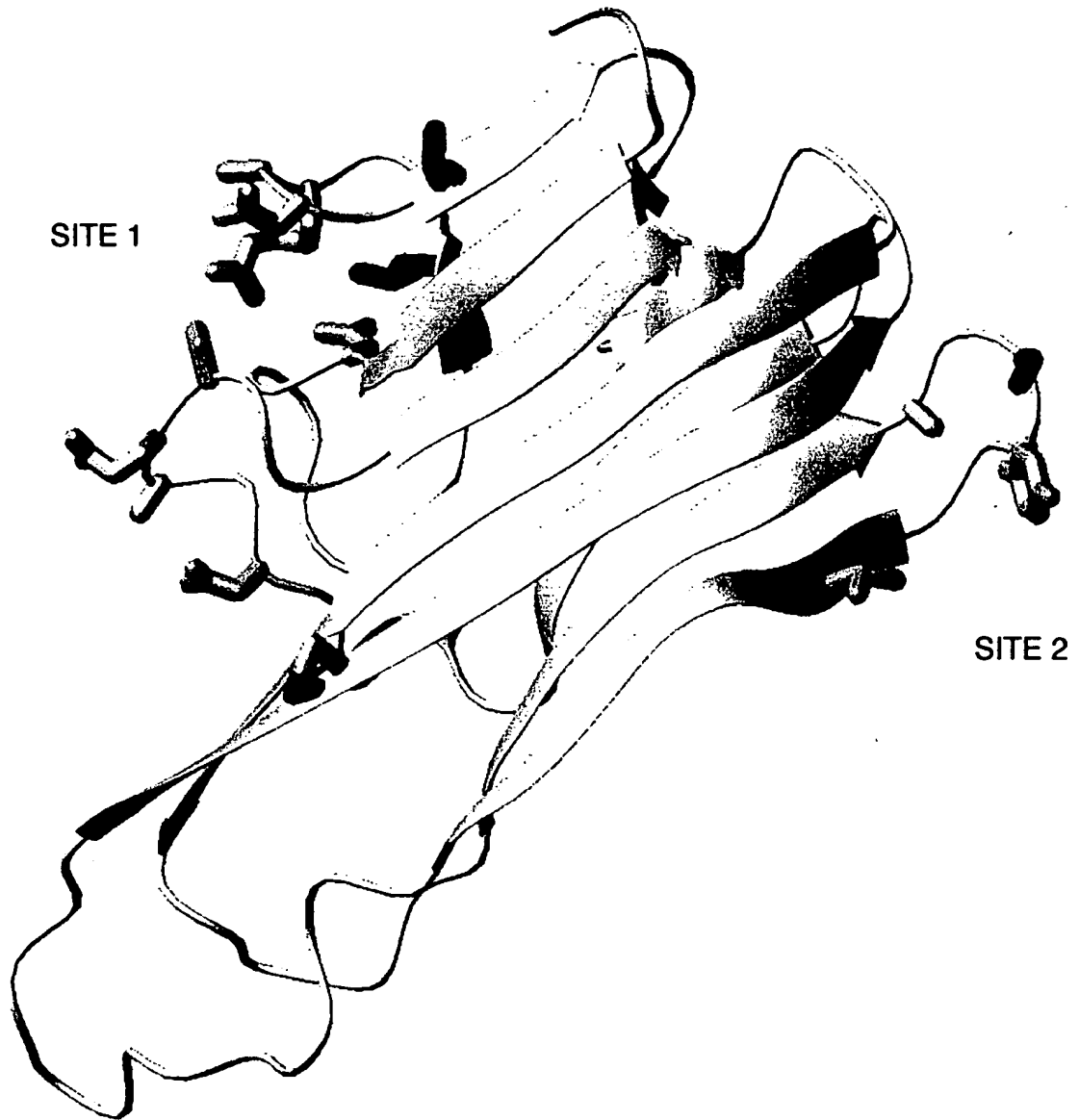


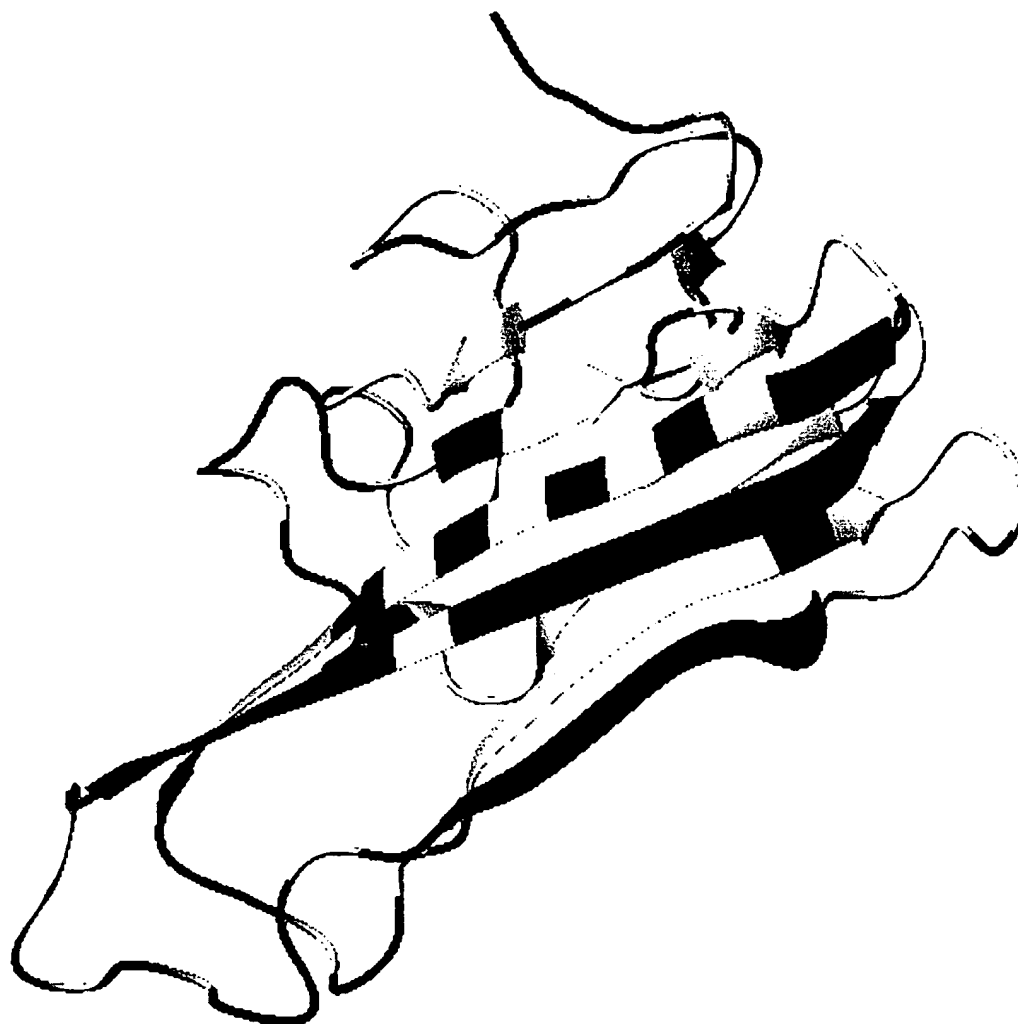
FIG..2

[illegible]

**FIG. 3**

TNF $\alpha$  BINDING SITES**FIG.\_4**

## TNF $\alpha$ TRIMER INTERFACE



**FIG. 5**

1 atgcaccacc accaccacca cgtacgctcc tcctcccga ctcggtccga caaacggta  
 61 gctcacgtag tagctaacc gcaggctgaa ggtagctgc agtggtgaa ccgccgcgt  
 121 aacgctctgc tggctaaccg tgtagaactg cgcgacaacc agctggtagt accgtccgaa  
 181 ggtctgtacc tgatctactc ccagggtactg ttcaaagggtc aggggtgtgc gtccactcac  
 241 gtactgtctga ctcacactat ctcccgcac gctgtatcct accagactaa agtaaacctg  
 301 ctgtccgcta tcaaattccc gtgtcagcgc gaaactccgg aagggtgtga agctaaaccg  
 361 tggtagaac cgatctacct ggggtgtgta ttccagctgg aaaaagggtga ccgctgtcc  
 421 gctgaaatca accgcccga ctacctggac ttcgctgaat ccggtcaggt atacttcggt  
 481 atcatcgctc tgtga

**FIG.\_6A**

1 MHHHHHHVRS SSRTPSDKPV AHVVANPQAE GQLQWLNRRR NALLANGVEL RDNQLVVPSE  
 61 GLYLIYSQVL FKGQGPCSTH VLLTHTISRI AVSYQTKVNL LSAIKSPCQR ETPEGAEAKP  
 121 WYEPIYLGGV FQLEKGDRLS AEINRPDYLD FAESGQVYFG IIAL

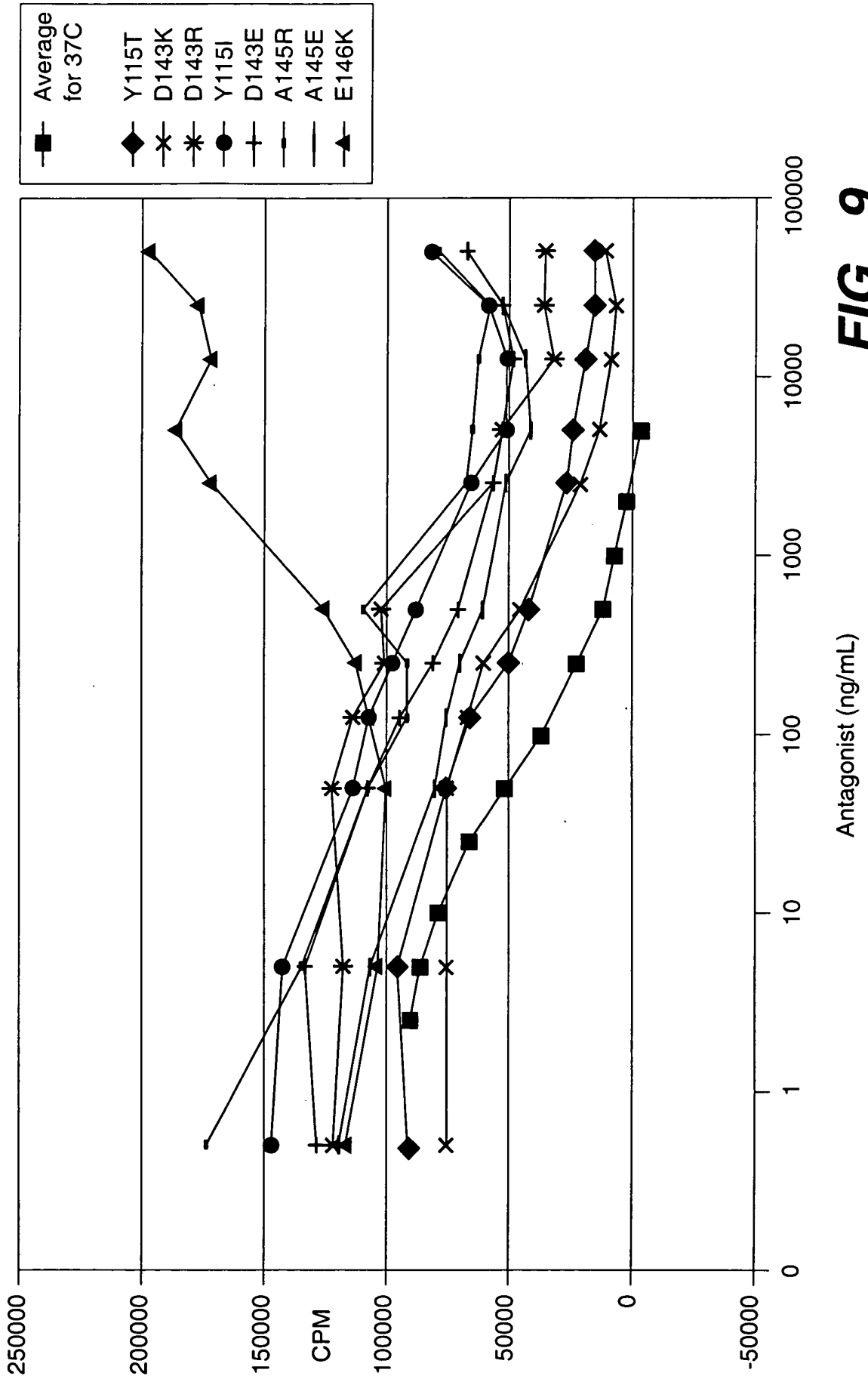
**FIG.\_6B**

Wild-type TNF amino acid	Wild-type TNF amino acid number	Mutants created
Q	21	R
N	30	D
R	31	I, D, E
R	32	D, E, S
A	33	E
A	35	S
K	65	D, T, M, W, I, Q, S, N, V, E
G	66	Q, K
Q	67	D, W, Y, R, K, S
A	111	R, E
K	112	D, E
Y	115	Q, K, E, N, R, F, H, M, L, I, W, D, T, S
D	140	R, K
D	143	E, N, Q, S, R, K
F	144	N
A	145	R, D, K, N, H, T, Q, E, Y, M, S, F
E	146	N, K, R, S
S	147	R

ALSO MADE DOUBLE MUTANTS K65E/D143K, K65E/D143R, K65D/D143K AND K65D/D143R

**FIG.\_7**





**FIG. 9**



Cell Death - Apoptosis

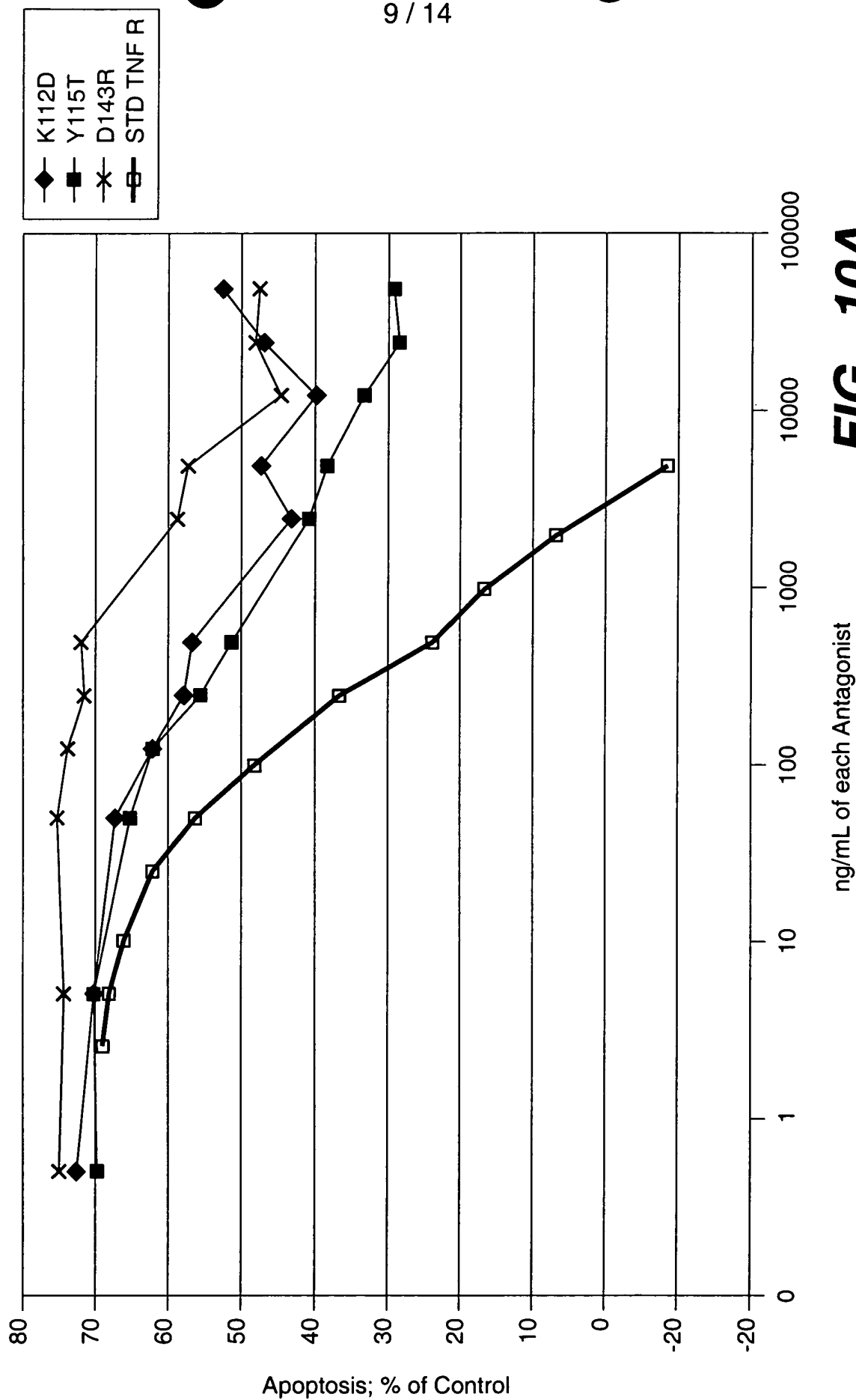


FIG. 10A

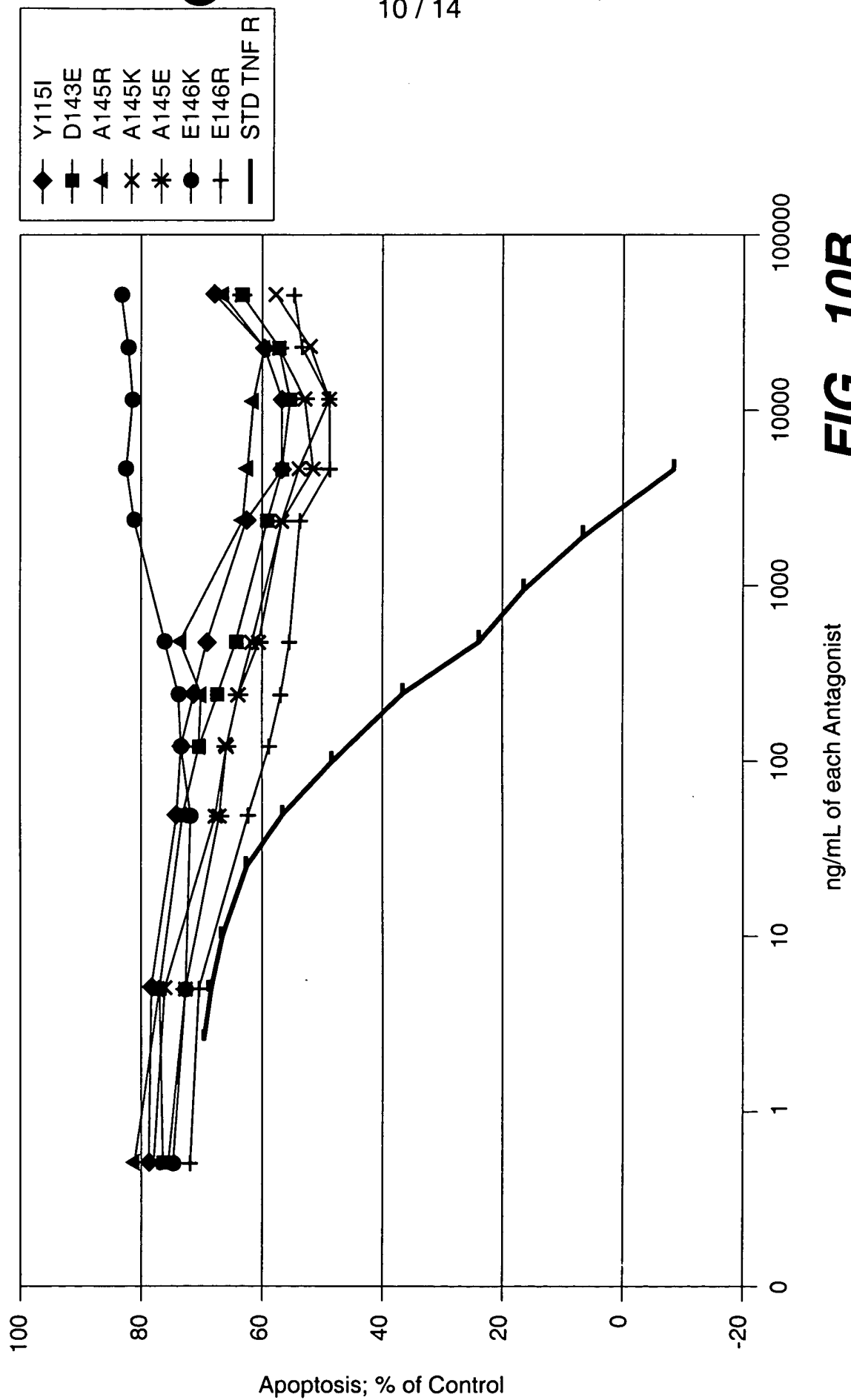


FIG. 10B



TRAF2(310-) DQDKIEALSSKVQQQLERSIGLKDLAMADLEQKVLEMEA STYDG

## FIG.\_12A

TRAF3(374-) VARNTGLLESQLSRHDQMLSVHDIRLADMDLRFQVLET ASYNG

## FIG.\_12B

TRAF5(343-) NDQRLAVLEEETNKHDTTHINIHKAQLSKNEERFKLLEG TCYNG

## FIG.\_12C

TRAF1(225-) DRERILSLEQRVVELQQTALQKDQALGKLEQSLRLMEE ASFDG

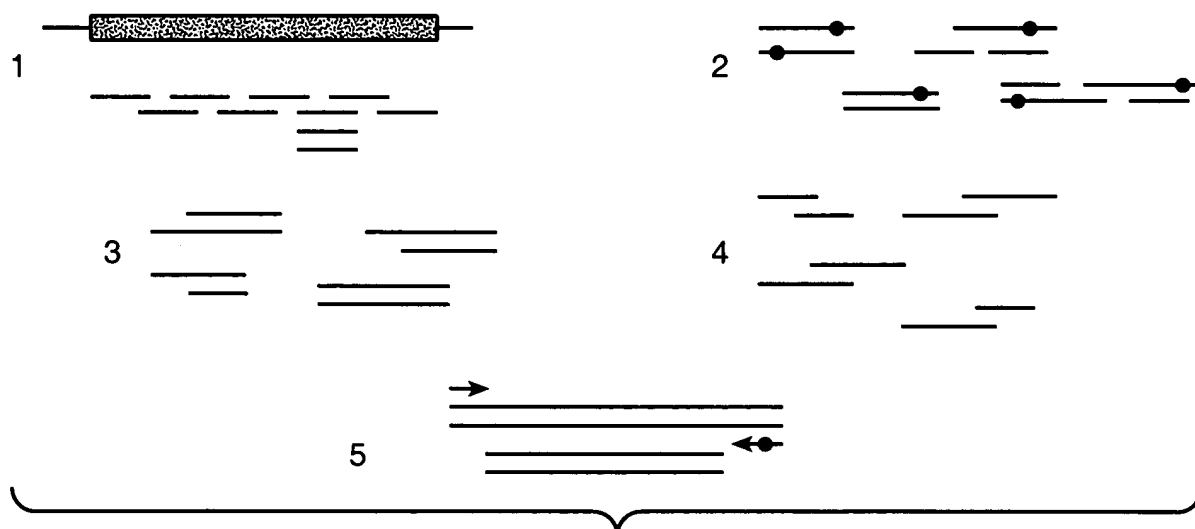
## FIG.\_12D

TRAF6(309-) QDHQIRELTAKMETQSMYVSELKRTIRTLEDKVAEIEA QQCNG

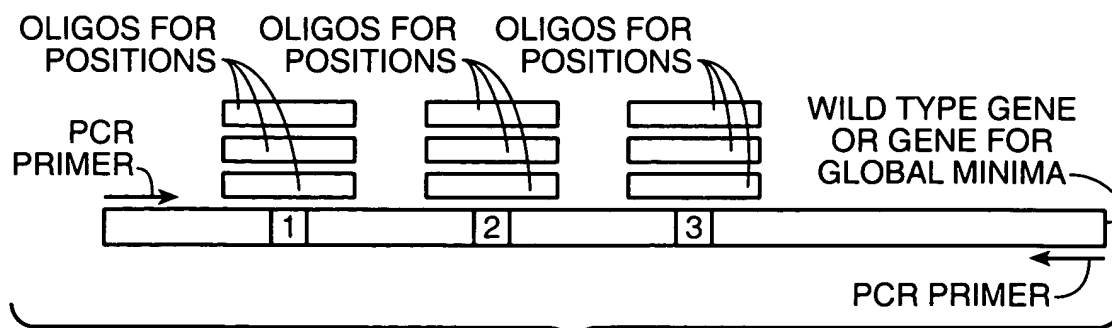
## FIG.\_12E

TRAF4(201-) -----CALVSRQRQELQELRRELEELSV GS-DG

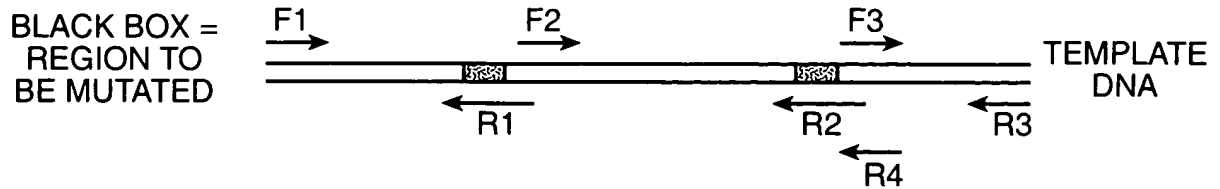
## FIG.\_12F



## FIG.\_13



## FIG.\_14



STEP 1: SET UP 3 PCR REACTIONS:

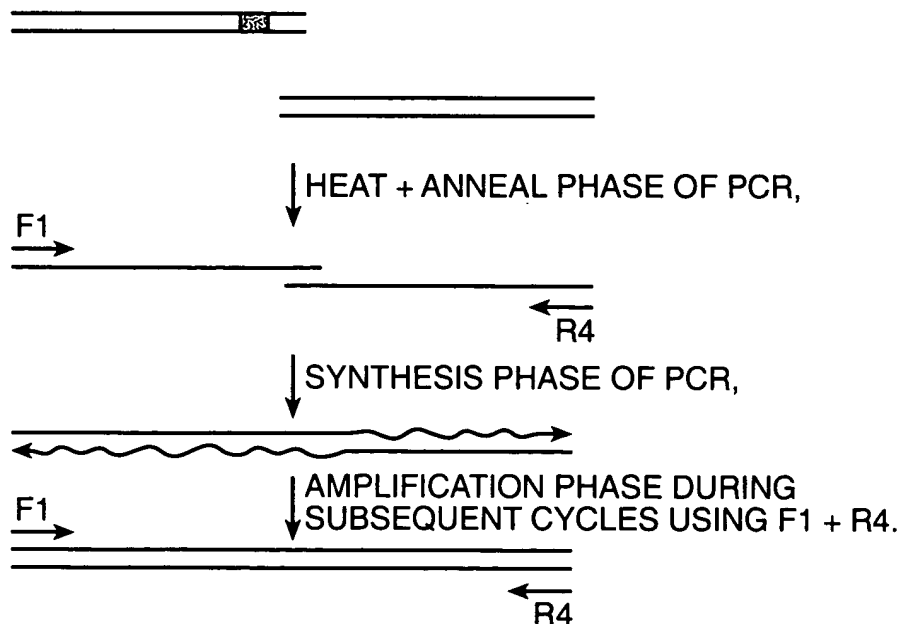
PRODUCTS:

TUBE 1:

TUBE 2:

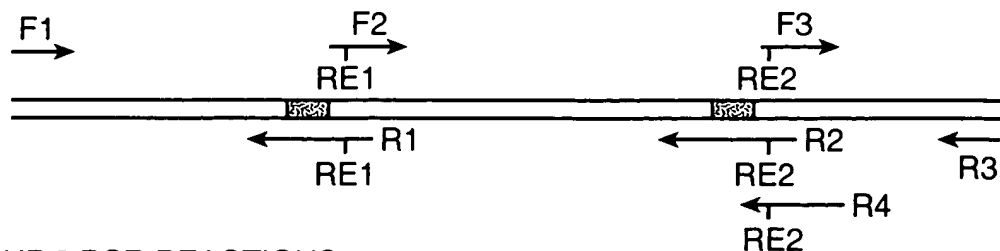
TUBE 3:

STEP 2: SET UP PCR REACTION WITH PRODUCTS OF TUBE 1 + PRODUCTS TUBE 2 + F1 + R4.



STEP 3: REPEAT STEP 2 USING PRODUCT FROM STEP 2 + PRODUCT FROM STEP 1, TUBE 3 + PRIMERS F1 + R3.

**FIG. 15**



**STEP 1:** SET UP 3 PCR REACTIONS:

**TUBE 1:** 
 A horizontal DNA template with a shaded box representing the RE1 site. Below the template, primer R1 is shown with an arrow pointing left.

**TUBE 2:** 
 A horizontal DNA template with two shaded boxes representing RE1 and RE2 sites. Below the template, primers R1 and R2 are shown with arrows pointing left. R1 is below RE1, and R2 is below RE2.

**TUBE 3:** 
 A horizontal DNA template with a shaded box representing the RE2 site. Below the template, primer R2 is shown with an arrow pointing left.

**STEP 2:** DIGEST PRODUCTS FROM STEP 1 WITH SUITABLE RESTRICTION ENDONUCLEASES.

**STEP 3:** LIGATE DIGESTED PRODUCT FROM STEP 2, TUBE 2 WITH DIGESTED PRODUCT FROM STEP 2, TUBE 1.



**STEP 4:** AMPLIFY VIA PCR LIGATED PRODUCTS OF STEP 3 WITH F1 + R4.



**STEP 5:** DIGEST AMPLIFIED PRODUCT OF STEP 4 WITH RESTRICTION ENDONUCLEASE #2.

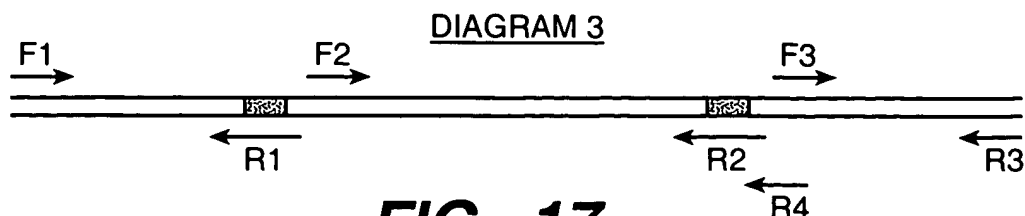


**STEP 6:** LIGATE PRODUCT FROM STEP 5 WITH PRODUCT FROM STEP 2, TUBE 1.



**STEP 7:** AMPLIFY PRODUCT FROM STEP 6 WITH F1 + R3.

**FIG. 16**



**FIG. 17**